

**July 9, 2020**

**Big Sky Wastewater Testing Results**

**Result Summary: Big Sky inflow sample was positive. Irrigation sample was negative.**

Sample Description:

- 1) A composite sample of wastewater (1.5 L total) inflow to the Big Sky treatment plant was captured on 7/7/2020 using an auto-sampler over the previous 24-hour period. Referred to below as “Inflow” samples in the **Table** below.
- 2) A single-catch sample (1.0 L total) of water used for irrigation of the Big Sky Golf Course was captured on 7/7/2020. Referred to below as “Irrigation” samples in **Table** below.

Testing Information and Raw Data:

Testing for the presence and abundance of the SARS-CoV2 genome in the above samples was performed using a kit designed by the US Centers for Disease Control and Prevention (CDC 2019-Novel Coronavirus (2019-nCoV), Real-Time RT-PCR Diagnostic Panel). Importantly, this test kit was originally designed to detect the virus in human samples and NOT wastewater or other kinds of environmental samples. The test was used here to determine whether a detectable amount of virus was present. Results need to be interpreted with caution, as described below.

Each of the above samples were split and processed as three replicates. Two tests were performed on each replicate and two independent locations on the SARS-CoV2 genome were targeted (N1 and N2). RNA was isolated from inactivated/concentrated samples, reverse-transcribed to DNA and used as template in quantitative PCR reactions as per kit instructions. Results were recorded as cycle threshold (Ct) numbers. All Ct numbers above (greater than) 40 cycles were highlighted in the table. Based on test interpretation guidelines described by the CDC (see below) Ct numbers greater than 40 should not be considered positive.

Results were as follows:

<b>Big Sky</b>				<b>Potential</b>
<b>Sample ID</b>	<b>Replicate ID</b>	<b>Target</b>	<b>Ct</b>	<b>Genomes per liter</b>
Inflow_1	Inflow_1.1	N1	33.12	11297
Inflow_1	Inflow_1.1	N2	34.52	9961
Inflow_1	Inflow_1.2	N1	32.54	18246
Inflow_1	Inflow_1.2	N2	34.90	7180
Inflow_2	Inflow_2.1	N1	32.69	16174
Inflow_2	Inflow_2.1	N2	34.35	11570
Inflow_2	Inflow_2.2	N1	32.38	20949
Inflow_2	Inflow_2.2	N2	34.33	11787
Inflow_3	Inflow_3.1	N1	32.82	14486
Inflow_3	Inflow_3.1	N2	34.20	13161
Inflow_3	Inflow_3.2	N1	33.24	10241
Inflow_3	Inflow_3.2	N2	35.34	4878

Irrigation_1	Irrigation_1.1	N1	NA	NA
Irrigation_1	Irrigation_1.1	N2	NA	NA
Irrigation_1	Irrigation_1.2	N1	NA	NA
Irrigation_1	Irrigation_1.2	N2	NA	NA
Irrigation_2	Irrigation_2.1	N1	NA	NA
Irrigation_2	Irrigation_2.1	N2	NA	NA
Irrigation_2	Irrigation_2.2	N1	NA	NA
Irrigation_2	Irrigation_2.2	N2	NA	NA
Irrigation_3	Irrigation_3.1	N1	NA	NA
Irrigation_3	Irrigation_3.1	N2	NA	NA
Irrigation_3	Irrigation_3.2	N1	NA	NA
Irrigation_3	Irrigation_3.2	N2	NA	NA

Interpretation:

Virus signal for both N1 and N2 targets was observed in all replicate inflow samples. All signals had Ct numbers below 40. These results are entirely consistent with a positive result. The trend in virus levels was increased from last week's sample, suggesting that the number of cases may be increasing. In contrast, no virus signal was observed in irrigation samples, suggesting that little to no virus is in irrigation water. No virus signal has been detected in irrigation water to date.

Relevant text from CDC guidelines:

"...a specimen is considered positive for 2019-nCoV if all 2019-nCoV marker (N1, N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct)."

"When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2 but not both markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is inconclusive."